Docket No.: 3691-0130PUS1
(Patent)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application of:	Koji SODE		
Application No.:	10/574,085	Confirmation No.:	8739
Filed:	April 16, 2007	Art Unit:	1652
For:	GLUCOSE DEHYDROGENASE/CYTOCHROME FUSION PROTEIN	Examiner:	T. Saidha

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Koji Sode, Ph.D. declare the following:

1. I am the inventor of the present application.

2. I have reviewed the Office Action dated January 28, 2011.

3. In support of the written description rejection and the enablement rejection, the Examiner cites Guo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 2004, 101:9205-9210 (Guo) for the proposition that the percentage of random single substitution mutations, which inactivate a protein (3-methyladenine DNA glycosylase) is 34% (x factor). The Examiner states that this number appears to be consistent for other proteins. The Examiner further asserts that the teachings in Guo indicate that to find a single active mutant, within random mutants having 95% identity to SEQ ID NO: 2, one of skill in the art would have to screen over several million mutants. The Examiner states that the level of unpredictability can only be imagined if the entire sequence of SEQ ID NO: 2 is modified as encompassed by the language of claim 1(b).

4. It is my opinion that an ordinary artisan at the time of the invention would not have had to resort to preparing and testing enormous numbers of SEQ ID NO: 2 variants to obtain those, which retain the desired functions. An ordinary artisan would have recognized from the present application and the art known at the time of the invention, which amino acids could have been deleted, substituted, or added in SEQ ID NO: 2, while concomitantly retaining glucose dehydrogenase activity and electron transfer capabilities. In addition, as described further below, Guo is not relevant to the fusion protein of SEQ ID NO: 2.

5. As described in the Examples of the originally filed application, SEQ ID NO: 2 is a fusion protein between pyrroloquinoline quinone glucose dehydrogenase (PQQGDH) from *Acinetobacter calcoaceticus* and a cytochrome domain from *Comamonastes testosteroni*. At the time of the invention, an ordinary artisan would have recognized that PQQGDH from *Acinetobacter calcoaceticus* is a well characterized enzyme. Further, amino acid residues had been identified at the time of filing, which have a role in, for example, substrate oxidation and/or substrate specificity, *see for example* Exhibit C11, *i.e.*, Igarashi *et al.*, "Engineering PQQ glucose dehydrogenase with improved substrate specificity site-directed mutagenesis studies on the active center of PQQ glucose dehydrogenase", *Biomol. Eng.*, April 2004, 21:81-9, including page 88, *of record*, and Exhibit C16, *i.e.*, Igarashi *et al.* "Molecular engineering of PQQGDH and its applications", *Arch. Biochem. Biophys.* August 1, 2004, 428:52-63, including page 58, Table 5, *of record.*

6. For example, Table 5 and the section entitled "[r]egion responsible for enzyme function" on page 58 of Exhibit C16 teaches that His168, Gln192, and Arg252 recognize glucose. Exhibit C16 further teaches that mutations at His168 show drastic decreases in catalytic activity and catalytic efficacy, *see* page 58 of Exhibit C16. Moreover, Table 5 of Exhibit C16 describes the regions and residues of PQQGDH associated with, among other properties, improved substrate specificity, but decreased catalytic activity, thermal stability, and broader substrate specificity.

7. Further, a variety of PQQGDH mutants derived from *Acinetobacter calcoaceticus* PQQGDH were available in the art before or shortly after the priority date of the present

invention, *see* Exhibits A1-A3, B1-B10, and C1-C16, *of record*. The mutants described in these documents comprise various amino acid deletions, substitutions, or additions. Nevertheless, the mutants retain glucose dehydrogenase activity and electron transfer ability.

8. In view of the art known at the time of the invention, it is my opinion that an ordinary artisan would have recognized those regions of PQQGDH and those amino acid residues, which are associated with catalytic activity and substrate specificity. Accordingly, an ordinary artisan would have been aware of the substitutions, deletions or insertions, which are likely to destroy the desired function as well as those mutations, which are likely to result in retention of activity. That is, an ordinary artisan was aware of and could envision the structure of the variants described in the claims, which would retain glucose dehydrogenase activity and electron transfer ability.

9. Further, an ordinary artisan would not have needed to test an enormous number of SEQ ID NO: 2 mutants to obtain a mutant having the desired activity. An ordinary artisan could have reasonably predicted from the art known at the time of the invention, which mutants would retain glucose dehydrogenase activity and electron transfer ability.

10. It is also my opinion that the teachings in Guo do not establish that the claims fail to comply with the written description requirement or the enablement requirement. The Guo document only calculates the "x factor" for AAG. Accordingly, the results described in Guo do not apply to SEQ ID NO: 2 of the present claims.

11. In particular, the authors in Guo state that "[w]e advance the concept of the x factor as a measure of protein tolerance to random substitutions....It may be of particular interest to examine x factors from various protein families and diverse organisms", *see* page 9201 of Guo, right column. The authors further discuss the effects of variable x factors, for example, when x = 1, *see* page 9209, right column of Guo.

12. In view of the Guo disclosure as a whole, it is my opinion that the Guo authors suggest that the x factor is not 34% for every amino acid sequence.

13. Moreover, as noted above, the structure-function relationship of PQQGDH and the specific amino acid residues involved in the catalytic activity and/or substrate specificity were already known in the art as evidenced by Exhibits A1-A3, B1-B10, and C1-C16. Thus, those of ordinary skill in the art could easily prepare a variety of mutants having glucose dehydrogenase activity and electron transfer ability, not by searching from an indefinite number of mutants as the Examiner asserts, but by selecting specific mutants based upon the knowledge of the structure-function relationship.

14. In view of the foregoing, it is my opinion that the specification adequately describes the SEQ ID NO: 2 variants. It is also my opinion that the experimentation required to obtain mutants of SEQ ID NO: 2, which demonstrate glucose dehydrogenase activity and electron transfer ability, would have been routine in the art at the time of the invention.

STATEMENT UNDER 18 U.S.C. § 1001

I declare that all statements made herein of my own knowledge are true and that all statements made herein on information are believed to be true. I further declare that the statements were made with knowledge that willful false statements and the like are punishable by fine or imprisonment or both under Section 1001, Title 18 of the United States Code.

Dated: _____ Respectfully submitted,

By_____
Koji Sode, Ph.D.